ORIGINAL ARTICLE

Comparison of release and transport of manure-borne *Escherichia coli* and enterococci under grass buffer conditions

A.K. Guber[1], J.S. Karns[2], Y.A. Pachepsky[2], A.M. Sadeghi[3], J.S. Van Kessel[2] and T.H. Dao[4]

1 Department of Environmental Sciences, University of California, Riverside, CA, USA
2 USDA-ARS Environmental Microbial Safety Laboratory, Beltsville, MD, USA
3 USDA-ARS Hydrology and Remote Sensing Laboratory, Beltsville, MD, USA
4 USDA-ARS Environmental Management and Byproduct Utilization Laboratory, Beltsville, MD, USA

Keywords
enterococci, *Escherichia coli*, infiltration, manure, overland transport, release, runoff.

Correspondence
Y.A. Pachepsky, USDA-ARS-BA-ANRI-EMSL, 173 Powder Mill Road, BARC-EAST, Beltsville, MD 20705, USA. E-mail: ypachepsky@anri.barc.usda.gov

2006/1052: received 20 July 2006, revised 25 August 2006 and accepted 19 September 2006

doi:10.1111/j.1472-765X.2006.02065.x

Abstract

Aim: To test the hypothesis that *Escherichia coli* and enterococci bacteria have similar release rates and transport characteristics after being released from land-applied manure.

Methods and Results: Turfgrass soil sod was placed into 200 cm long boxes that had the top two 25 cm sections separated to monitor the release and infiltration of bacteria, which affected bacteria transport in the rest of the box. Dairy manure with added KBr was broadcast on the top two sections. Boxes with either live or dead grass stand were placed under a rainfall simulator for 90 min. Runoff and infiltration samples were collected and analysed for Br, *E. coli*, enterococci and turbidity. Significant differences in release kinetics of *E. coli* and enterococci were found. A change from first-order release kinetics to zero-order kinetics after 1 h of rainfall simulation was observed.

Conclusion: Differences in release rates but not in the subsequent transport were observed for *E. coli* and enterococci.

Significance and Impact of the Study: Because both *E. coli* and enterococci are currently used as indicator organisms for manure-borne pathogens, the differences in their release rates may affect the efficiency of using these organisms as indicators.

Introduction

Escherichia coli and enterococci are common indicator organisms used to detect potential faecal contamination of surface and ground water and to determine water quality (USEPA 2000; COM 2002). Concentrations of these organisms in surface and ground waters depend on both their ability to be transported from the contamination source in the overland flow and in the subsurface and their survival in environments encountered during transport (Jamieson *et al.* 2002; Ferguson *et al.* 2003).

Coupled monitoring of both organisms has demonstrated differences in dynamics of their concentrations in waters that were reached by faecal contamination. Personné *et al.* (1998) observed differences in transport of *E. coli* and enterococci in a karst subsurface. The much earlier appearance of enterococci in groundwater wells when compared with *E. coli* was attributed to the better mobility of enterococci in unsaturated soil. Ramos-Cormenzana *et al.* (1994) observed that enterococci possessed better resistance to dryness and an excellent ability to move with the infiltrating water when compared with *E. coli*. Celico *et al.* (2004) suggested that faecal coliforms and faecal enterococci vary considerably in terms of size, morphology, motility and surface chemistry, which lead to substantive differences in their propensities for attachment to solid surfaces within soils and aquifers. Becker *et al.* (2003) observed that coccidial (spherical shaped) bacteria were transported in fractured bedrock better than all but one strain of rod-shaped bacteria.

Little, if anything is known about the differences in transport characteristics of the two indicator organisms in soil, or about the differences in their transport in overland flow. If differences in the transport of *E. coli* and enterococci exist, this may substantially affect the efficiency of using these organisms as indicators of faecal contamination from agricultural lands.

The objective of this work was to test the hypothesis that *E. coli* and enterococci have similar patterns of release from animal faeces and subsequent transport in the environment.

Materials and methods

The bacteria release and transport experiments in this work consisted of an application of bovine manure with added bromide salt on grass-covered soil in experimental boxes, followed by continual sampling of the runoff water and water infiltrated through soil during the simulated rainfall. Concentrations of *E. coli*, enterococci, bromide, and turbidity were determined in the collected water samples.

Soil boxes

Six soil boxes with 200 cm × 41 cm × 10 cm dimensions (Fig. 1), wooden sides and plywood bottoms were used in this experiment. The boxes were waterproofed by caulking the seams and painting the overall interior and exterior with boat-sealing paint. Each box was divided into five sections (marked by letters 'a' to 'e', in Fig. 1a,b) with four vertical blades installed at the bottom at distances of 25, 50, 100 and 150 cm from the upper side of each box. The first blade was 4·5 cm high, and all others were 1 cm high. Each section had a hole in the bottom centre near the blade (Fig. 1a). Teflon funnels (1–5 in Fig. 1b) were mounted into each hole to collect infiltrating water. A 5 cm layer of commercial turfgrass sod (J. T. Patton and Sons, Poolesville, MD, USA) was carefully cut to the box size and placed in the box. Soil in section 'a' became separated from the rest of the layer, as the high first blade cut it off. A small trough with the rain shield ('S' in Fig. 1c) was placed on the top of the first blade to collect the runoff from section 'a'. A larger trough ('L' in Fig. 1c) was mounted at the box bottom to collect runoff from the rest of the soil. The space between the edges of the soil layer and the interior box walls was filled with polyurethane foam sealant (Great Stuff, M#162848, Dow Chemical Co., Midland, MI, USA). Soil in the boxes was clay loam and had the saturated hydraulic conductivity of $2{\cdot}2 \pm 1{\cdot}1$ cm h^{-1}.

Boxes with soil were stored outdoors under a screen to prevent direct solar radiation, and were manually irrigated daily to keep the grass intact before the experiments started. Funnels were open to allow drainage. The grass was manually clipped to an average height of 7·5 cm and clippings were removed before each run of experiments. Three experimental runs were made with the live grass in three of the boxes. Grass was then allowed to wilt completely in the other three experimental boxes, and another three rainfall simulation runs were made with the dead grass conditions.

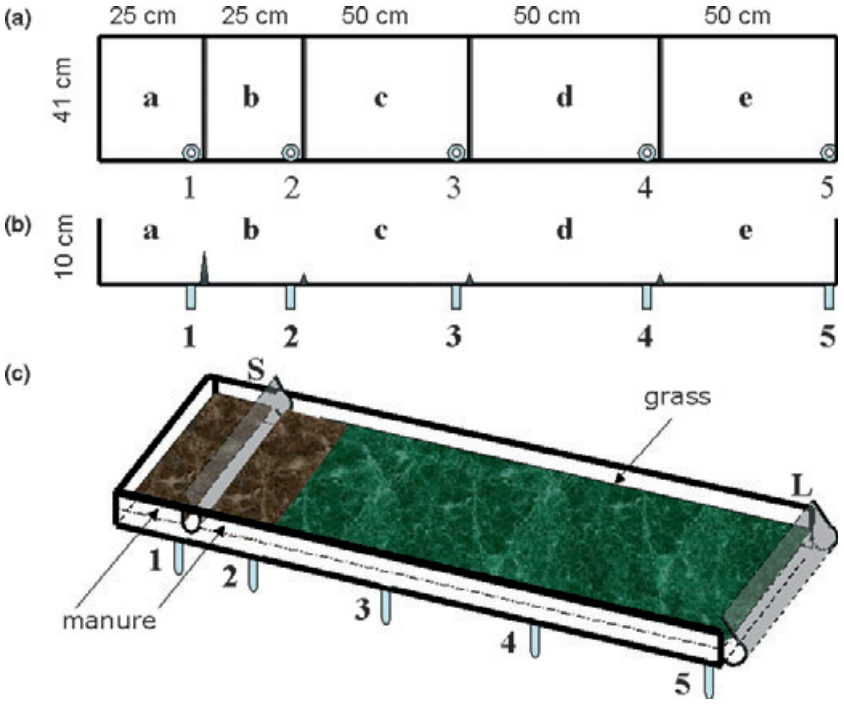

Figure 1 Soil box design and manure application areas. (a) Top view of the empty soil box; a–d, box sections separated by blades (═) protruding from the bottom; (◉) teflon infiltration funnels. (b) Side view of the empty box, (▲) bottom blades; 1–5, teflon infiltration funnels, same as in (a). (c) Soil box with grass sod and manure applied over sections 'a' and 'b' of the box; 1–5, teflon infiltration funnels; 'S' and 'L', troughs to collect surface runoff.

Grass in the boxes was tall fescue (*Festuca arundinacea*). Plant densities and biomass, and surface roughness were determined after experiments; data are available upon request.

Rainfall simulation

Manure slurry was collected from a free-stall barn at the Dairy Research Unit of the USDA-ARS-Beltsville Agricultural Research Center (BARC), Beltsville, MD housing lactating Holstein dairy cows (*Bos taurus*), thoroughly mixed and stored at 4°C overnight prior to the runoff experiments.

In each of the experimental runs, one soil box was placed under the rain simulator and tilted to have a 4% slope. Rainfall was applied using a rain simulator equipped with a TeeJet^{TM} 1/4 HH SS 14 WSQ nozzle (Spraying Systems Co., Wheaton, IL, USA) mounted on a frame at 3 m above the soil surface. Simulated rainfall had the uniformity coefficient of 0·93 ± 0·02 within the box placement area. Rainfall intensity of 3·24 ± 0·06 cm h^{-1} was maintained. Such rainfall has an approximate 1-year return frequency in Maryland. Soil within the box was first saturated using the rainfall simulator for approximately 30 min and allowed to drain for an additional 30 min prior to manure application. This was carried out to minimize the hydrological variability related to antecedent moisture and to collect background runoff and infiltration samples. The irrigation water was prepared by adding laboratory grade chemicals to the deionized water to obtain a typical Maryland rainwater composition. Concentrations of Ca, Mg, K, Na, NH_4, NO_3, Cl and SO_4 were 0·08, 0·03, 0·02, 0·12, 0·34, 1·36, 0·26 and 1·9 mg l^{-1}, respectively, pH was 4·5.

To use Br as a conservative tracer present only in the liquid phase of the manure, sterile KBr solution was added to the manure and was manually stirred for 3 min. Concentration of KBr in manure was 0·3 g l^{-1}. Manure was then applied uniformly on the upper 25 cm 'a' and 'b' sections of each experimental box (Fig. 1) at a rate of 11·7 kg m^{-2} (about 3·5 times higher than a typical agronomic rate), and rainfall was initiated. Runoff and infiltration samples were collected from troughs 'S' and 'L' and from funnels 1–5 (Fig. 1), first after the runoff or infiltration initiation in each of the collection points, and then at times equal to the multiples of 5 min (e.g. 10, 15, 20 min, etc.), counting from the rainfall initiation. The rainfall simulations continued for 90 min.

Microbiological and chemical analyses

Enterococci in water samples were enumerated by plating a 50 μl exponential spiral onto plates of mEnterococcus agar (Difco Labs, Detroit, MI, USA) using an Autoplate 4000 spiral-plater (Spiral Biotech, Gaithersburg, MD, USA) and counting red colonies that developed after 48 h incubation at 37°C with a Q-Count colony counter (Spiral Biotech, Norwood, MA, USA). Numbers of *E. coli* in water were determined by plating 50 μl exponential spirals on MacConkey agar and counting red colonies that developed after overnight incubation at 44·5°C. The number of enterococci and *E. coli* in manure was determined by plating as above after dilution with sterile water. Each value was the average of two plates.

Bromide content was measured with a Model 9635BN Bromide Ion-Selective Electrode (Thermo Electron Corporation, Beverly, MA, USA) in the detection range from 0·4 to 79 900 ppm. Turbidity was determined using a 2020 Turbidimeter (LaMotte Company, Chestertown, MD, USA) with the Tungsten incandescent bulb as the light source. The turbidimeter was calibrated for raw manure measurements in the range from 0 to 40 000 mg manure l^{-1}, and values of nephelometric turbidity units (NTU) in the raw manure were evaluated using the calibration coefficients and measurements in 1: 10 manure dilution. Manure samples (100 g) were oven-dried at 60°C to measure the solid:liquid ratio.

Results

Table 1 summarizes concentrations of *E. coli*, enterococci, Br and turbidity in manure, irrigation water, and background infiltration samples from funnels 1–5 obtained prior to manure application. Background micro-organism concentrations were four to six orders of magnitude lower than concentrations in manure. The solid: liquid ratio in manure was 0·08, and pH was 8·02 with 1: 10 manure dilution.

The runoff collected in the large trough constituted from 0% to 16% and from 14% to 23% of the irrigation water applied over the box sections 'b', 'c', 'd' and 'e' in the live-grass boxes and the dead-grass boxes, respectively. The amount of runoff in the small trough varied from 8% to 38% and from 21% to 99% of the irrigation water applied over the box section 'a' in the live-grass boxes and in the dead-grass boxes, respectively.

Data in Fig. 2 show the temporal dependencies of concentrations of *E. coli*, enterococci, Br and turbidity in both runoff and infiltration water in experiments with live grass. The data from section 'a' demonstrate the two-stage kinetics of the manure constituents' release. The first stage of the exponential decrease of micro-organism concentrations continued for about 40 min. After that, the decrease in concentrations was much slower. Funnel 2 was expected to mimic funnel 1, and this was observed. All constituent concentrations at funnel 3, which was

located at 50 cm from the manure application zone, increased during the first 20 min and then decreased with time at about the same rate as concentrations at funnel 2. Concentrations at funnel 4 were much smaller than in funnel 3 and approached nearly to the background level. The distance between funnels 3 and 4 was only 50 cm, but it appeared to be sufficient to provide dilution of bromide such that the Br concentrations approached the level in the rainwater. The leachate from funnel 5 also had concentrations mostly lower than leachate from funnel 4, and they were closer to the background concentrations. In general, funnel 5 had concentrations higher than the large trough.

Figure 3 shows the concentrations and turbidity in runoff and infiltration from the manure patch in live-grass experiments. A significant difference was observed in release rates of *E. coli* and enterococci (Table 2), whereas no significant difference was found between the release rates of Br and *E. coli*. Results similar to the data in Table 2 were found in experiments with the dead grass (data not shown).

The dead- and live-grass experiments were compared using the analysis of variance (ANOVA). The vegetation status was the significant ($P < 0{\cdot}05$) factor along with sampling time and sampling location. At logarithmic scale, there was a small but statistically significant difference of about 0·3 between average relative *E. coli* concentrations in experiments with live and dead grass. The same was found for enterococci. No such effect was found for Br. Comparison of concentrations at the release points (i.e. small trough, funnels 1 and 2) showed that the concentrations at the second release stage (last 20 min) were significantly less in dead-grass experiments compared with the live-grass experiment.

Discussion

Dilution and loss to infiltration were the dominant mechanisms for the decrease in micro-organism and tracer concentrations in runoff during their transport from the applied manure. Concentrations of all manure constituents tended to be lower in the runoff than in infiltrating water. The rainfall diluted the water running off the manure surface and laterally moved out the liquid part of the manure that could infiltrate. Concentrations in funnel 3 were also affected by the lateral transport in soil (Fig. 2). The delay in the concentration growth in funnel 3 when compared with funnel 2 reflected the time needed for the suspended manure components to pass through the soil and arrive with the runoff water to section 'c' where funnel 3 collected the infiltrated water. The low level of all the manure constituent concentrations in funnel 4 indicated that the concentrations in this funnel were mainly

Table 1 *Escherichia coli*, enterococci and bromide concentrations, and turbidity measured in manure, irrigation water and leachate from soil prior to manure application

Source	*Escherichia coli* (CFU ml^{-1})		Enterococci (CFU ml^{-1})		Bromide (ppm)		Turbidity (NTU)	
	Live grass	Dead grass	Live grass	Dead grass	Live grass	Dead grass	Live grass	Dead grass
Manure	$1{\cdot}07 \times 10^6$ ± $0{\cdot}38 \times 10^6$	$1{\cdot}79 \times 10^6$ ± $0{\cdot}78 \times 10^6$	$0{\cdot}77 \times 10^6$ ± $0{\cdot}46 \times 10^6$	$0{\cdot}50 \times 10^6$ ± $0{\cdot}31 \times 10^6$	316 ± 108	2553 ± 94	$8{\cdot}04 \times 10^4$ ± $1{\cdot}47 \times 10^4$	$4{\cdot}88 \times 10^4$ ± $2{\cdot}31 \times 10^4$
Irrigation water	0	0	0	0	0·26 ± 0·06	0·12 ± 0·09	0·62 ± 0·11	0·51 ± 0·10
Background leachate	8·86 ± 3·80	8·33 ± 16·34	355 ± 172	173 ± 183	0·58 ± 0·19	0·71 ± 0·19	114 ± 185	94 ± 264

(Average ± SD).

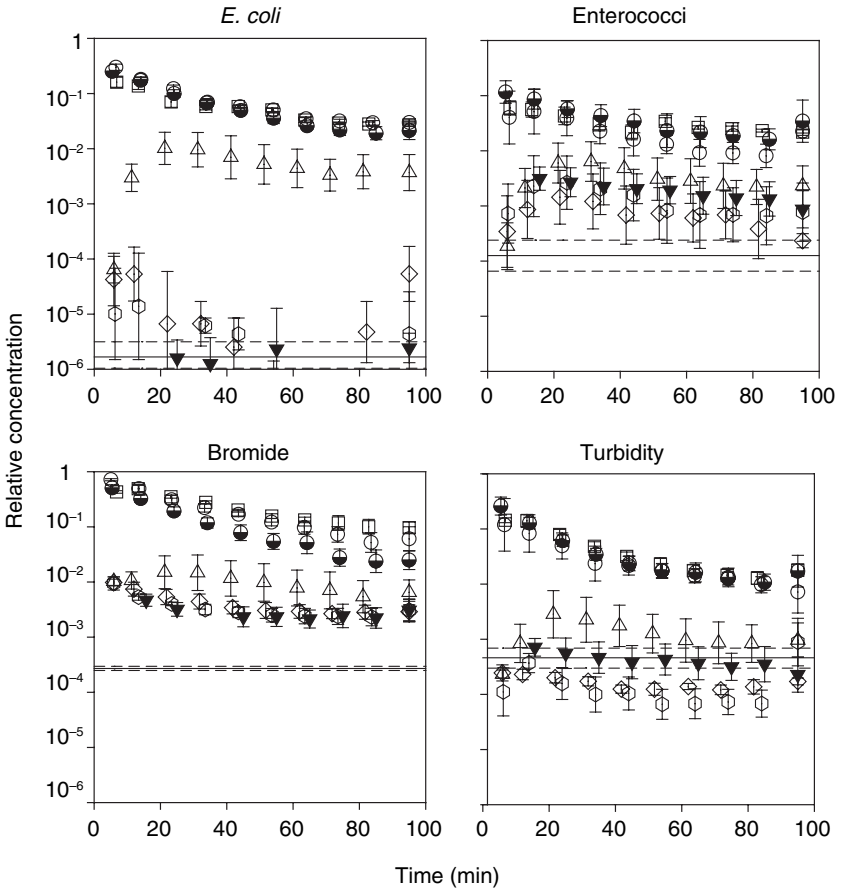

Figure 2 Effluent concentrations of bacteria, bromide ion and turbidity in experiments with live grass; (◒) small trough (S in Fig. 1); (○, □, △, ◇ and ⬡) infiltration funnels 1–5 in Fig. 1, respectively; (▼) large trough (L in Fig. 1). The error bars show the SEs of geometric means from triplicated experiments. Solid and dashed lines show the geometric average ± SE of the background concentrations.

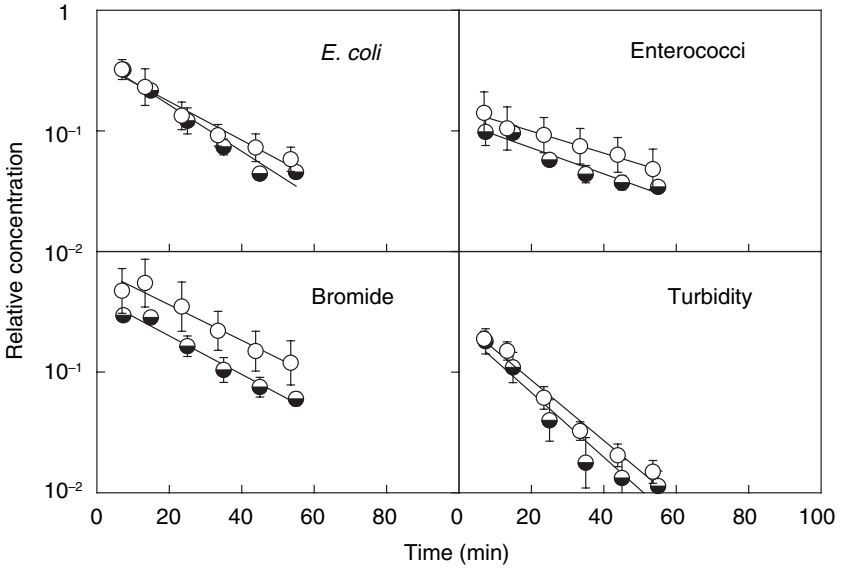

Figure 3 Bacteria, bromide, and turbidity in runoff (◒) and infiltrated water (○) from the section 'a' in Fig. 1 in triplicated live-grass experiments; linear regression lines are also shown.

controlled by transport in runoff followed by infiltration of the runoff water, rather than by transport through the soil matrix. This could be deduced from the fact that bromide did not interact with soil, and *E. coli* and enterococci demonstrated a behaviour similar to that of bromide. The concentrations in funnels 1 and 5 located under troughs were lower than concentrations in the corresponding troughs 'S' and 'L'. This was explained by the additional

Table 2 Release rates (min^{-1}) of manure constituents

Released manure constituent	Runoff	Infiltration
Enterococci	0·0248[a]	0·0210[a]
Escherichia coli	0·0443[b]	0·0368[b]
Br	0·0368[b]	0·0339[b]
Turbidity	0·0617[c]	0·0576[c]

The same superscript letter means that the average values do not differ significantly at the significance level of 0·05.

dilution of the surface runoff with the rainwater when compared with infiltrating suspensions.

Substantial differences in the release kinetics of manure constituents shown in Fig. 3 and Table 2 could be attributed to the distribution of these constituents between readily and sparsely leachable parts of manure. Bromide was added to the liquid part of manure. The similarity in bromide and *E. coli* release kinetics indicated that *E. coli*, like Br, resided in the liquid part of manure, and was released as the liquid manure fraction was diluted and displaced by the rainwater. Enterococci were apparently present in substantial numbers in less readily suspended, possibly solid parts of manure. The difference between the relative concentrations of *E. coli* and enterococci in the first runoff and infiltration water samples supported this conjecture (Figs 2 and 3). The average relative concentrations of Br, *E. coli* and enterococci in the first sample in funnel 1 were 0·50, 0·25 and 0·14, respectively. This could also be caused by the presence of enterococci in the part of manure that was less available for leaching when compared with *E. coli* and Br.

After the first 40 min of rainfall, the release of enterococci and *E. coli* occurred with zero-order kinetics (Fig. 2). The gradual dissolution of manure lumps probably occurred without a significant decrease in the size of the lumps, bringing more or less constant concentrations of enterococci in the runoff and infiltration solutions. These data indicate that a 30-min rainfall simulation, recommended, for example, for the phosphorus release studies (e.g. Tarkalson and Mikkelsen 2004), may not be sufficient to properly observe and evaluate the kinetics of manure-borne bacteria release. In particular, estimating the first-order kinetics rate from the 0·5 h release data may create a distorted (too optimistic) forecast of the concentrations in the runoff.

We could not separate effects of the vegetation status and temporal differences in manure consistence and composition, because experiments with dead grass were carried out 1 month later than experiments with live grass. Variations of manure properties affected the relative turbidity that was significantly higher in live- than in dead-grass experiments (data not shown). Differences revealed with ANOVA were probably related to the temporal differences in manure consistence and composition rather than to the differences in the vegetation status, because the vegetation status was not a significant factor for bromide concentrations.

Our data do not show differences in the transport characteristics of the two organisms, because the 1·5-m distance appeared to be sufficient to bring the concentrations of the released organisms in runoff and infiltration water to the background levels. Such differences may be more pronounced in experiments where infiltration is less dominant, e.g. either soil is sealed or soil water content is high. The small depth of soil layer would have a substantial impact on our results compared with a field situation. First, there is only a 5 cm depth of soil in which infiltration, and therefore filtration of bacteria by the soil matrix, can occur. Secondly, the shallow depth of soil would have restricted the infiltration process via the impermeable boundary presented to vertical flow at the base of the box. This might have caused water pressure to 'back up' to the surface, thereby increasing the ratio of runoff to infiltration, compared with a continuous column of soil in the field. Thirdly, the sod cutting probably created some disturbance of soil. Some possible precautions were taken, such as cutting sod to the width of boxes, avoiding rolling the sod, and allowing soil to settle in boxes under live grass for a month with daily irrigation. Nevertheless, the sod may have been disturbed, although the similarity in concentrations shown with the error bars in Fig. 2 indicates relatively homogeneous soil layers. Various environmental factors may affect or control transport of released manure constituents in field conditions. For example, rainfall intensity and manure type are known to affect manure leaching (Schijven *et al.* 2004). Vegetated buffer strips can provide a filtering effect on runoff containing suspended material, and this effect may vary depending on plant status (Dabney *et al.* 1995). It remains to be seen whether some discernible differences in transport of *E. coli* and enterococci exist in field conditions.

Overall, our data demonstrate the differences in release behaviour of two water quality indicator organisms – *E. coli* and enterococci from manure to the grassed soil surface. The concentrations at the source of release need to be monitored to discern the differences in organisms' release from differences in their transport.

Acknowledgement

We are grateful to the anonymous reviewers whose comments and suggested amendments have been consequential for the manuscript improvement.

References

Becker, M.W., Metge, D.W., Collins, S.A., Shapiro, A.M. and Harvey, R.W. (2003) Bacterial transport experiments in fractured crystalline bedrock. *Ground Water* **41**, 682–689.

Celico, F., Varcamonti, M., Guida, M. and Naclerio, G. (2004) Influence of precipitation and soil on transport of fecal enterococci in fractured limestone aquifers. *Appl Environ Microbiol* **70**, 2843–2847.

COM (2002) *Proposal for a Directive of the European Parliament and of the Council Concerning the Quality of Bathing Water*. 581 Final, 24/10/2002. Brussels: Commission of the European Communities, 2002/0254 (COD).

Dabney, S.M., Meyer, L.D., Harmon, W.C., Alonso, C.V. and Foster, G.R. (1995) Depositional patterns of sediment trapped by grass near Stephenville hedges. *Trans ASAE* **38**, 1719–1729.

Ferguson, C., de Roda Husman, A.M., Altavilla, N., Deere, D. and Ashbolt, N. (2003) Fate and transport of surface water pathogens in watersheds. *Crit Rev Environ Sci Technol* **33**, 299–361.

Jamieson, R.C., Gordon, R.J., Sharples, K.E., Stratton, G.W. and Madani, A. (2002) Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: a review. *Can Biosyst Eng* **44**, 1.1–1.9.

Personné, J.C., Poty, F., Vaute, L. and Drogue, C. (1998) Survival, transport and dissemination of *Escherichia coli* and enterococci in a fissured environment. Study of a flood in a karstic aquifer. *J Appl Microbiol* **84**, 431–438.

Ramos-Cormenzana, A., Castillo, A., Incerti, C. and Gomez-Palma, L.F. (1994) Bacteriological indicators of faecal contamination: result of a loading experiment with untreated urban water. *J Appl Bacteriol* **76**, 95–99.

Schijven, J.F., Bradford, S.A. and Yang, S. (2004) Release of *Cryptosporidium* and *Giardia* from dairy cattle manure: physical factors. *J Environ Qual* **33**, 1499–1508.

Tarkalson, D.D. and Mikkelsen, R.L. (2004) Runoff phosphorus losses as related to soil test phosphorus and degree of phosphorus saturation on piedmont soils under conventional and no-tillage. *Commun Soil Sci Plant Anal* **35**, 2987–3007.

USEPA (2000) *Implementation Guidance for Ambient Water Quality Criteria for Bacteria*. 1986. Draft. January 2000. EPA 823/D-00-001. Washington, DC: U.S. Environmental Protection Agency, Office of Water.

